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Report Title

Automated Sample Preparation (ASP)

Development of a rapid method to sequentially isolate nucleic acids and protein from any sample type by a cartridge-based system

ABSTRACT

CUBRC has developed an in-line, multi-analyte isolation technology that utilizes solid phase extraction chemistries to purify and concentrate nucleic acids and protein for downstream analysis. This technology will be automated by developing a single use, cartridge-based unit. Development of such a unit is critical to reaching the US Government's and Department of Defense's biological threat countermeasure development goals. Specifically, CUBRC will design and manufacture a prototype cartridge(s) and test the prototype cartridge for its ability to isolate each analyte individually and in succession. Testing will be performed on both laboratory derived samples and samples typical of those generated by aerosol collectors.

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Number of Manuscripts:

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Patents Submitted

Patents Awarded

Awards

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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FTE Equivalent:

Total Number:

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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FTE Equivalent:

Total Number:

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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Total Number:

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
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Steven Gertz	1.00	
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Total Number: **1**

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This section only applies to graduating undergraduates supported by this agreement in this reporting period

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Total Number:

Names of other research staff

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Technology Transfer



ARO Contract #: W911NF-11-C-0100

Automated Sample Preparation (ASP)

Development of a rapid method to sequentially isolate nucleic acids and protein from any sample type by a cartridge-based system

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Foreword

CUBRC has developed an in-line, multi-analyte isolation technology that utilizes solid phase extraction chemistries to purify and concentrate nucleic acids and protein for downstream analysis. This technology will be automated by developing a single use, cartridge-based unit. Development of such a unit is critical to reaching the US Government's and Department of Defense's biological threat countermeasure development goals. Specifically, CUBRC will design and manufacture a prototype cartridge(s) and test the prototype cartridge for its ability to isolate each analyte individually and in succession. Testing will be performed on both laboratory derived samples and samples typical of those generated by aerosol collectors. The goal of this program is to advance CUBRC's multi-analyte sample preparation technology into a single-use cartridge format. This program will design, manufacture and test a prototype cartridge for sequential nucleic acid and protein isolation from a sample.

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Statement of the problem studied

CUBRC, Inc. has developed a rapid method to sequentially isolate pure nucleic acids and protein from virtually any sample type. This technology is extremely important for both the detection and diagnostic capabilities of the US Government and Department of Defense. The democratization of advanced science and technology combined with the threat posed by terrorism and rogue nations has led to an urgent need to develop appropriate countermeasures to threats posed by biological agents, including natural, emerging and engineered agents. Biological threat agents are comprised of a large number of diverse organisms leading to unique and challenging problems when developing countermeasures. One of these problems is correct identification or diagnosis of a particular threat agent. There have been extraordinary advances made in the detection, identification and diagnostic fields in response to this recognized problem. Despite these advances, barriers to a complete set of countermeasures remain. One such barrier is posed by the apparent disconnect between the identifier or diagnostic device and the actual sample to be tested. This disconnect can be seen when highly sensitive identifiers or diagnostic devices fail to recognize an agent because samples contain interferents or inhibitors that prevented proper function of the molecular tools employed by the identification device. In an attempt to remove interferents and inhibitors, many device developers have determined a preparation step is required to remove interferents and inhibitors prior to testing the sample in their devices.

Sample preparation refers to the isolation of an analyte or macromolecule class such as nucleic acids from the sample milieu. Sample preparation presents an identifier or diagnostic device with clean analytes which in turn improves the sensitivity and accuracy of the technology. There are a large number of sample preparation technologies and devices on the market today, however most have one or more limitations that prevent them from being widely accepted by the communities charged with their use[1-3]. Typically, the nucleic acid isolation kits produced by Qiagen are considered the industry standard however they are designed for laboratory use, require laboratory tools, and are not well suited for field or high-throughput applications. CUBRC, Inc. has developed a sample preparation technology that overcomes these limitations. Our sample preparation technology is based on the well-known BOOM® chemistry that isolates nucleic acids by a silica-based solid state mechanism[4]. We have developed a similar solid-phase extraction chemistry to purify protein from a sample following nucleic acid isolation[5]. CUBRC's sample preparation technology is unique in that both the nucleic acid and protein content of a sample can be isolated sequentially. Also unique to our technology is the form factor of the solid phase extraction matrix. Both solid phase extraction chemistries utilize a silica-based matrix that has been placed into a pipette tip. In this form, the user simply pipettes the sample through the matrix. Depending on the chemistries used, either the nucleic acid or protein content of the sample selectively binds to the silica matrix. The sample can be passed through the matrix multiple times to increase binding with the matrix. The form factor of our silica matrix eliminates the need for bulky or sensitive laboratory equipment and requires only a pipettor. This form factor also reduces the time needed for isolation, as nucleic acids can be isolated in under 5 minutes and both nucleic acids and protein in under 20 minutes. Importantly, because the matrix is simply built into a cylindrical fitting and liquid flowed over it, it is very amenable to automation.

Automation of our analyte isolation technologies is a straightforward process that is progressing along two paths. The first path is to build single-use cartridges that will process a sample, without user intervention, for the two analyte types. This cartridge will be fit into a device containing all of the necessary software and pumps to drive the fluidics for sample processing. The second path is to simply fit the matrix into pipette tips that are designed for high throughput robotics platforms. These platforms could rapidly isolate samples for triage or confirmatory analysis in hospital or mobile laboratory settings. This program will design, manufacture, and test a prototype cartridge thus advancing development of the automated cartridge platform beyond its current breadboard configuration.

Macromolecule Isolation Performance Data: *Nucleic Acid Isolation:* The nucleic acid isolation procedure is performed using a form modification of the well-characterized chaotropic salt/silica chemistry (BOOM® chemistry). As stated previously, the form modification is the utilization of a cylindrical pipette with a silica-based capture matrix embedded within. This form factor is known as the TruTip™. Captured nucleic acids are eluted from the matrix with the provided elution buffer or any buffer preferred by the user. Generally, the elution volume (100-250 µL) is smaller than the sample volume, leading to a concentration of the target analyte; nucleic acids in this case. The TruTip™ method is compatible with all known elution buffers (typically aqueous solutions) and therefore can accommodate the requirements of any

Bacteria

B. anthracis (veg) @ 10³ CFU/mL

Extraction Method	Results	Avg. CT
Qiagen	30/30 positive	28.3 ± .3
TruTip	30/30 positive	28.1 ± .7

Virus

VEEV @ 10⁴ PFU/mL

Extraction Method	Results	Avg. CT
Qiagen	30/30 positive	31.3 ± .5
TruTip	30/30 positive	30.8 ± .6

Table 1: Head to head, 30/30 comparison of the TruTip extraction process against the Qiagen standard. Bacteria (upper) & virus (lower) were spiked into blood at the stated concentration.

using the TruTip™ protocol and another 30 samples were processed using the Qiagen Genomic DNA kit. The data show that both kits extracted PCR-ready target DNA from all samples and that the amount of DNA, as measured by the average CT, was identical. A similar experiment was performed using Venezuelan equine encephalitis virus (VEE virus) at a concentration of 10⁴ PFU/mL in whole blood. In this case, the Qiagen Viral RNA kit was required whereas a common TruTip™ protocol was employed for comparison. As with the previous experiment, the data show an identical amount of PCR-ready nucleic acid was isolated. Overall, these experiments

downstream detection or diagnostic device. After a short set-up procedure where the user measures out reagents into the proper tubes, the user need only pipette for multiple cycles in the proper buffer to extract PCR-ready nucleic acids.

Nucleic acid isolation using the TruTip™ is the best characterized and tested of the TruTip™ capabilities. In head-to-head evaluations the TruTip™ technology was shown to positively ID assorted bacteria and viruses as reliably as the current industry gold-standard, Qiagen. The data in Table 1, upper and lower panels, were obtained using Qiagen kits specific to the organism of interest whereas the TruTip™ protocol was identical for each organism. These data show that the single TruTip™ protocol was as efficient as the Qiagen kits at extracting PCR ready nucleic acids from a sample. The data in the upper panel was obtained using vegetative *Bacillus anthracis* spiked into whole blood at a concentration of 10³ CFU/mL. Thirty samples were processed

indicate that the TruTip™ method extracts nucleic acids for PCR-analysis with equal efficiency to the industry gold standard, requires significantly less time per sample, can be applied to all viral and bacterial targets studies without adjusting the protocol, and does not require cumbersome laboratory equipment.

The TruTip™ nucleic acid isolation technology has been developed to be compatible with a multitude of sample matrices. The list below identifies the compatible matrices that have been identified to date:

- Water / Tris buffer
- Swabs with buffer
- Sputum
- Nasal wash
- Whole blood
- Soil
- Urine
- Saliva
- Paraspinal fluid

For all of the identified matrices, the standard TruTip™ nucleic acid isolation procedure worked well however some adjustments may improve the yield from each sample type. For instance, a preliminary homogenization step for sputum or soil matrices has been shown to improve the flow of the sample through the capture matrix thus maximizing the flow rate while increasing the capture efficiency.

The TruTip™ nucleic acid extraction technology has been developed into a prototype integrated sample preparation/identification cartridge for use with the TruDx suite of instruments from Akonni Biosystems (figure 1). This prototype cartridge is under evaluation by Akonni Biosystems and has performed admirably compared to the handheld, TruTip™ unit, outperforming the handheld units in many cases. The design of the sample preparation portion of this prototype will be incorporated into the CUBRC prototype.

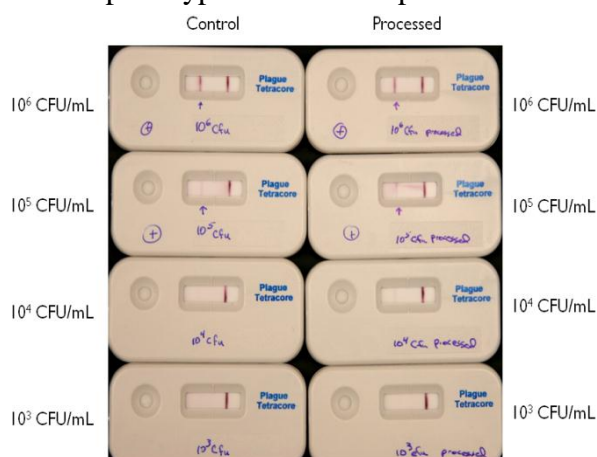


Figure 1: Hand-Held Assays from Tetracore. The left column is unprocessed sample while the right column where identical samples processed using the TruTip protein isolation protocol.

To conclude, the TruTip™ nucleic acid isolation procedure is a simple, yet robust method to isolate nucleic acids both in the lab and in the field. The procedure is compatible with multiple matrices and all pathogenic organisms. The design of the TruTip™ has made it extremely amenable to automation and compatible with cartridge development.

Protein Isolation: The TruTip™ nucleic acid & protein isolation technology is the only marketed kit capable of sequentially isolating both protein and nucleic acids. The “waste” from the nucleic acid isolation is added to the protein isolation buffer. This buffer is an alcohol solution that strips the shell of hydration from the protein within the sample. Our alcohol based buffer is

similar in principle to the ethanol precipitation of protein from guanidine solutions described by Pepinsky [6]. The protein is isolated by binding to a second silica matrix. Using the identical silica capture matrix material for protein and nucleic acid isolation simplifies the product for the user while reducing logistical burdens.

CUBRC has also demonstrated the field deployability of the handheld version of this technology by isolating nucleic acid and protein from *Yersinia pestis* (avirulent A1122 strain) samples and identifying the resultant protein analyte on a *Y. pestis* specific hand-held assay from Tetracore (Figure 1). The data from the hand-held assays show that the samples isolated using the TruTip™ isolation process were able to identify *Y. pestis* at a concentration of 10^4 CFUs which is an order of magnitude lower than that in samples that were not processed. These data speak to the TruTip™ isolation method's ability to concentrate and purify the analyte of interest-protein in this case.

To conclude, the protein isolation technique performed sequentially, post-nucleic acid isolation, allows the user to test multiple analyte types from the same sample without splitting the sample.

Benefit to the Warfighter: The development of a simple, robust technology that isolates two distinct macromolecule classes from most matrices for downstream identification and analysis is critical for today's Warfighter. As the Warfighter is confronted with the challenges posed by biological warfare and terrorism, new methods to rapidly "detect to protect" are needed. Sample preparation is a critical step in this pathway. By simplifying the isolation technologies and removing the majority of user handling steps, the CUBRC automated sample preparation (ASP) system provides a rapid, reliable and standardized process for biological threat agent sensing. By providing multiple types of macromolecules for analysis, the CUBRC ASP system provides the Warfighter a measure of redundancy not seen in any other system in today's market.

Summary of the most important results

The CUBRC team has designed and built an automated nucleic acid and protein isolation device,

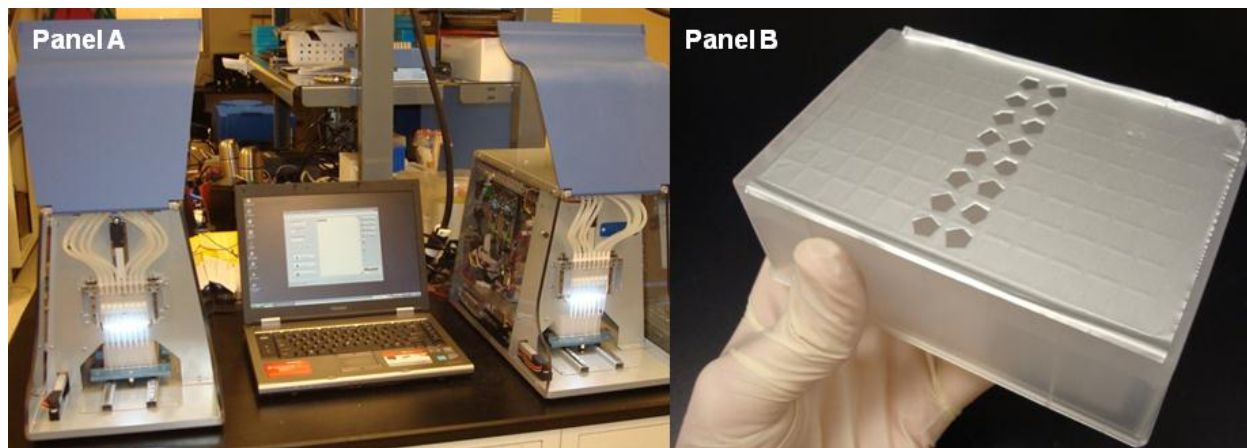


Figure 2: Panel A shows two '380' devices with the Laptop control in the center. The control software is open on the laptop screen. Panel A shows a cartridge with punctured foil cover. The '380' manipulates the cartridge to the proper position aligning solid phase extraction tips to the correct well. The '380' lowers the tips into the wells and draws up and aspirates the contents.

the ‘380,’ and has developed single use cartridges to process eight samples, simultaneously. The ‘380’ has been developed from concept stage through computer aided design to the bechtop prototypes shown in figure 2. The biomarker isolation chemistries and protocols have been fully developed. In addition, protocols have been written into the software developed specifically to control the ‘380.’ This software is compatible with the Windows XP and Windows 7 operating systems and provides the user with a simple “point-and-click” method of operation. The protocols that we are using to isolate nucleic acids followed by protein from a sample are similar to the protocol that we’ve developed and published for our hand-held extraction device [7]. Our protocols we’re converted to a text format compatible with the ‘380’ software (Appendix C). Without going into detail, there are a number of variables that are manipulated by the ‘380’ which are controlled from a single interface and fairly simple alter as necessary.

During testing, we did find a weakness in the ‘380’ design. The attachment nozzles for the solid phase extraction tips broke on three occasions. The nozzles were redesigned and a new manifold assembled. This new design has performed well, with no breakages and identical extraction data to the original (see 2012 Q3 DTRA report).

Multi-analyte extraction, DNA isolation analysis:

The results for comparative DNA extractions of *Bacillus globigii* spores using three devices have been analyzed by qrtPCR and reported in table 2 below. These results were obtained from 3 separate triplicate extractions, meaning each data point was from determined by the average of 9 total samples. We also utilized two different buffers, Joint Portal Shield Buffer (JPSB) and our in-house Automated Sample Preparation buffer (ASP buffer).

Table 2: Results of comparative nucleic acid extraction from *B. globigii* spores

						"380"				TruTip				DNAPro				
Spores		JPSB Lysate		ASP Lysate		JPSB		ASP buffer		JPSB		ASP buffer		JPSB		ASP buffer		
Cfu input	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)	avg cfu (% ST Dev)	avg ct (% ST Dev)
10 ⁷	1.52E+07 (29.70)	31.12 (5.52)	1.08E+07 (25.29)	31.34 (7.87)	8.76E+06 (27.83)	31.93 (4.97)	6.21E+10 (128.43)	22.50 (38.73)	1.01E+11 (128.83)	22.19 (38.35)	4.52E+04 (38.69)	39.69 (1.69)	3.04E+05 (58.86)	36.88 (2.28)	2.45E+06 (72.7)	35.35 (2.48)	1.07E+06 (92.2)	37.07 (5.18)
10 ⁶	2.44E+06 (73.59)	33.97 (5.63)	7.19E+05 (59.18)	35.76 (5.05)	6.87E+05 (55.70)	35.78 (6.27)	2.86E+10 (199.08)	27.62 (36.11)	3.69E+10 (141.28)	23.76 (34.14)	6.12E+04 (86.05)	39.63 (4.07)	2.52E+04 (47.71)	40.58 (1.69)	3.55E+06 (98.76)	36.60 (11.97)	3.13E+04 (57.33)	41.34 (2.04)
10 ⁵	1.24E+05 (42.76)	37.93 (2.73)	1.17E+05 (36.06)	38.27 (4.53)	7.03E+04 (59.57)	39.19 (5.51)	5.93E+10 (67.98)	23.43 (53.77)	2.38E+11 (115.32)	25.21 (64.35)	5.20E+03	42.86	1.60E+04 (41.48)	41.26 (1.69)	9.11E+05 (220.2)	41.28 (9.59)	3.24E+03	44.21
10 ⁴	1.62E+04 (32.58)	41.19 (2.08)	1.17E+04 (15.61)	41.58 (3.39)	7.70E+03 (32.12)	42.23 (4.36)	1.04E+09 (169.31)	30.33 (34.38)	4.67E+10 (173.01)	25.72 (30.29)					7.31E+03 (75.25)	43.41 (2.38)	3.67E+03	44.37

The qrtPCR results proved very interesting. Importantly, all of the controls (white columns) were very consistent and tightly clustered with standard deviations of approximately 5%. The ‘380’ system (yellow columns) appeared to perform very well in terms of isolating and concentrating DNA as the calculated recovered DNA was far higher than the input DNA concentration. However, it is important to note that the consistency of extraction was poor as can be seen by the standard deviations for Ct values ranging from 30-50% of the average value. The handheld TruTip device (green columns) performed consistently (Ct standard deviations averaging 2-2.5%) in this study however the TruTip did not isolate the amount of input DNA nor

did it concentrate the DNA. Finally, the DNAPro (blue columns), performed consistently in both the amount of DNA isolated as a function of input DNA and the standard deviation as a function of Ct value. One important note is that the performance could be affected by the buffer solution. For instance, the DNAPro appears to perform better when the diluent was Joint Portal Shield Buffer. This relationship did not appear to hold true for the ‘380’ or the TruTip where there was no statistically relevant difference.

Multi-analyte extraction, protein isolation analysis:

The results of three dot blot extractions performed in triplicate are tabulated as a percentage of positive identifications at each *Bacillus globigii* spore lysate titer in the table below.

Table 3: Comparative protein extraction results as identified by dot-blot analysis

	10⁷ CFU/mL	10⁶ CFU/mL	10⁵ CFU/mL	10⁴ CFU/mL
Spores in PBS (control)	100	100	50	0
Spore Lysate in JPSB (control)	100	100	100	0
Spore Lysate in ASP Buffer (control)	100	100	0	0
“380” / JPSB	100	100	100	100
“380” / ASP buffer	100	100	33	67
TruTip / JPSB	100	100	100	67
TruTip / ASP buffer	100	100	100	67
DNAPro / JPSB	100	100	100	100
DNAPro / ASP	100	100	100	100

These results show that the protein isolated using the “380” automated system is “concentrated” minimally 10-fold and likely more so. The chemistries that we developed for protein isolation are clearly amenable to the handheld devices as well. In particular, the chemistries work well with the DNAPro handheld device which is an electricity-free extraction tool. It is important to note that, due to volume constraints, the “380” system uses only 40% of the input that the handheld devices use. Thus, the drop-off in detection efficiency using the “380” with ASP buffer may simply due to less antigen supplied to the system and not a deficiency in the system’s ability to isolate the antigen.

When taken together, these data show that the ‘380’ system in conjunction with JPSB provides the most sensitive results though somewhat less consistent when using qrtPCR. The ‘380’ paired with JPSB also provides very consistent results and increased sensitivity for protein detection assays when coupled with nucleic acid isolation. In the final analysis, the ‘380’ system provides an excellent platform for the dual extraction of nucleic acids and proteins from a sample, thus enabling the warfighter with increased confidence of results through redundant identification tools.

Appendix A

Quantitation Report 1

Experiment Information

Run Name	BG pre-extracted 4-23-12
Run Start	4/24/2012 1:41:48 PM
Run Finish	4/24/2012 3:31:21 PM
Operator	
Notes	
Run On Software Version	Rotor-Gene 6.0.31
Run Signature	The Run Signature is valid.
Gain FAM/Sybr	5.
Gain JOE	5.
Gain ROX	5.
Gain Cy5	5.

Quantitation Information

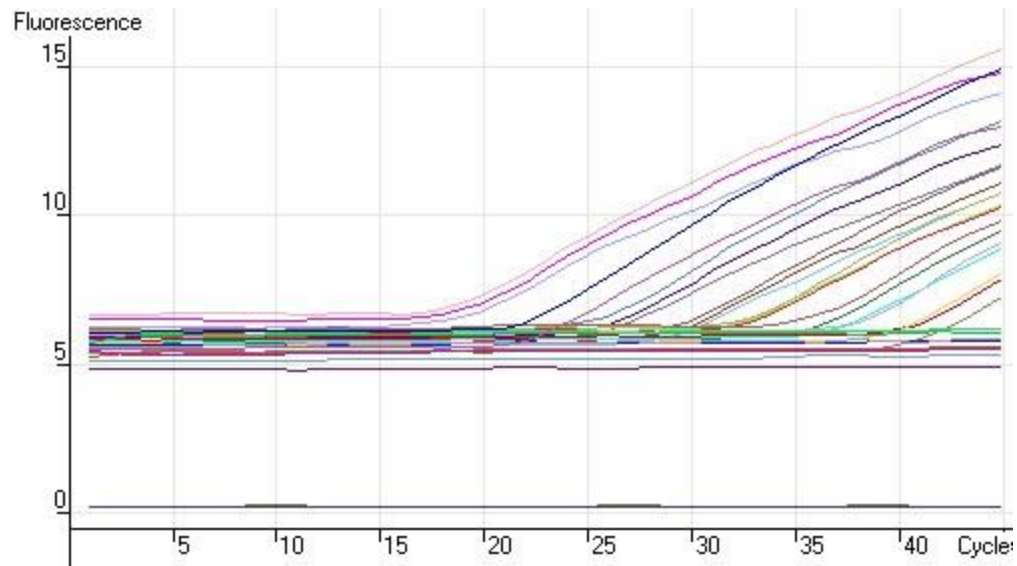
Threshold	0.01903
Left Threshold	7.000
Standard Curve Imported	No
Standard Curve (1)	$\text{conc} = 10^{(-0.288 \cdot \text{CT} + 13.038)}$
Standard Curve (2)	$\text{CT} = -3.476 \cdot \log(\text{conc}) + 45.328$
Reaction efficiency (*)	0.93931 (* = $10^{(-1/m)} - 1$)
M	-3.47649
B	45.32798
R Value	0.98631
R^2 Value	0.97281
Start normalising from cycle	1
Noise Slope Correction	Yes
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
No Template Control Threshold	10%
Sample Page	Page 1

Profile

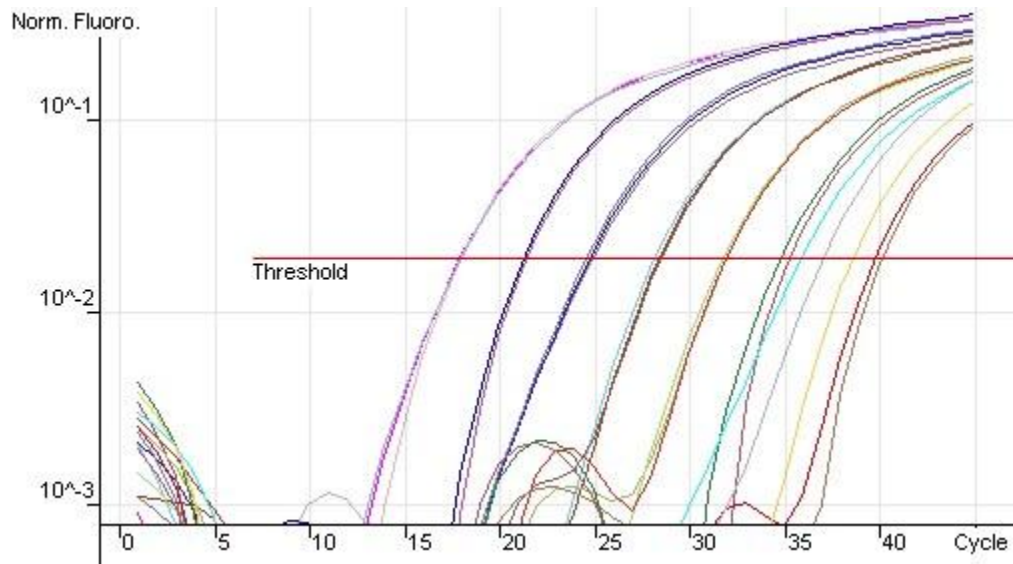
Cycle	Cycle Point
Hold @ 95°C, 10 min 0 secs	

Cycling (45 repeats)	Step 1 @ 95°C, hold 10 secs
	Step 2 @ 55°C, hold 45 secs, acquiring to Cycling A(Cy5,FAM/Sybr,JOE,ROX)

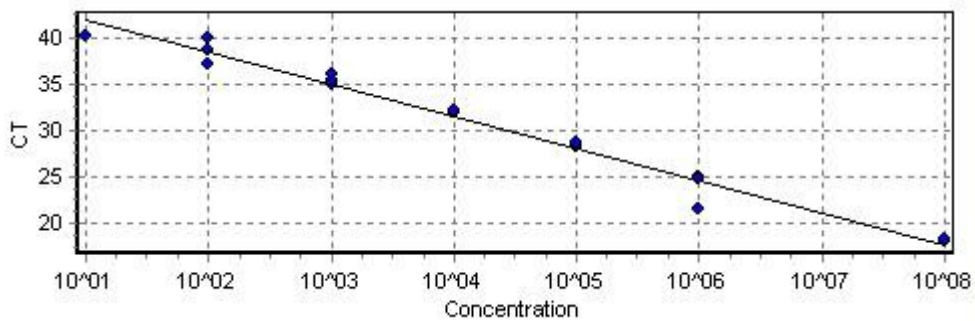
Raw Data For Cycling A.FAM/Sybr


































Quantitation data for Cycling A.FAM/Sybr



Standard Curve



No.	Colour	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
1		10 ⁸ Bg	Standard	17.99	100,000,000	72,884,307	27.1%
2		10 ⁸ Bg	Standard	18.03	100,000,000	70,951,063	29.0%
3		10 ⁸ Bg	Standard	18.07	100,000,000	69,334,863	30.7%
4		10 ⁶	Standard	24.97	1,000,000	718,646	28.1%
5		10 ⁶	Standard	24.85	1,000,000	779,005	22.1%
6		10 ⁶	Standard	24.66	1,000,000	881,548	11.8%
7		10 ⁵	Standard	28.25	100,000	82,004	18.0%
8		10 ⁵	Standard	28.57	100,000	66,084	33.9%
9		10 ⁵	Standard	28.49	100,000	69,735	30.3%
10		10 ⁴	Standard	32.05	10,000	6,618	33.8%
11		10 ⁴	Standard	31.84	10,000	7,558	24.4%
12		10 ⁴	Standard	32.00	10,000	6,808	31.9%
13		10 ³	Standard	35.34	1,000	744	25.6%
14		10 ³	Standard	34.89	1,000	1,005	0.5%
15		10 ³	Standard	35.95	1,000	499	50.1%
16		10 ²	Standard	39.87	100	37	62.8%
17		10 ²	Standard	38.68	100	82	18.2%
18		10 ²	Standard	37.11	100	231	131.4%
19		10 ¹	Standard		10		
20		10 ¹	Standard	40.21	10	30	196.7%
21		10 ¹	Standard		10		
22		1	Standard		1		
23		1	Standard		1		
24		1	Standard		1		
25		Unknown10 ⁷	Standard	21.36	1,000,000	7,863,223	686.3%

No.	Colour	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
27		10 ⁷	Standard	21.50	1,000,000	7,165,360	616.5%
28		NTC	Unknown				
29		NTC	Unknown				
30		NTC	Unknown				
31		NPC	Unknown				
32		NPC	Unknown				

This report generated by Rotor-Gene Real-Time Analysis Software 6.0 (Build 31)

Appendix B: Quantitation Report 2

Experiment Information

Run Name	BG extract 7-28-12
Run Start	8/29/2012 12:07:46 PM
Run Finish	8/29/2012 1:58:05 PM
Operator	
Notes	
Run On Software Version	Rotor-Gene 6.0.31
Run Signature	The Run Signature is valid.
Gain FAM/Sybr	5.
Gain JOE	5.
Gain ROX	5.
Gain Cy5	5.

Quantitation Information

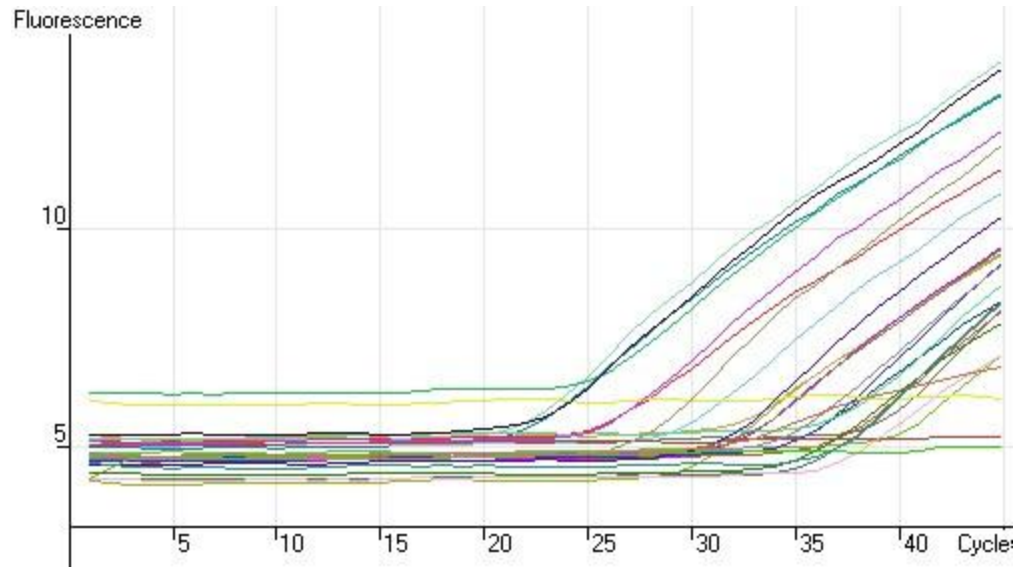
Threshold	0.0052
Left Threshold	7.000
Standard Curve Imported	No
Standard Curve (1)	$\text{conc} = 10^{(-0.335 \cdot \text{CT} + 14.311)}$
Standard Curve (2)	$\text{CT} = -2.987 \cdot \log(\text{conc}) + 42.741$
Reaction efficiency (*)	$1.16186 (* = 10^{(-1/m)} - 1)$
M	-2.98661
B	42.74073
R Value	0.9861
R^2 Value	0.9724
Start normalising from cycle	1
Noise Slope Correction	Yes
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
No Template Control Threshold	10%
Sample Page	Page 1

Profile

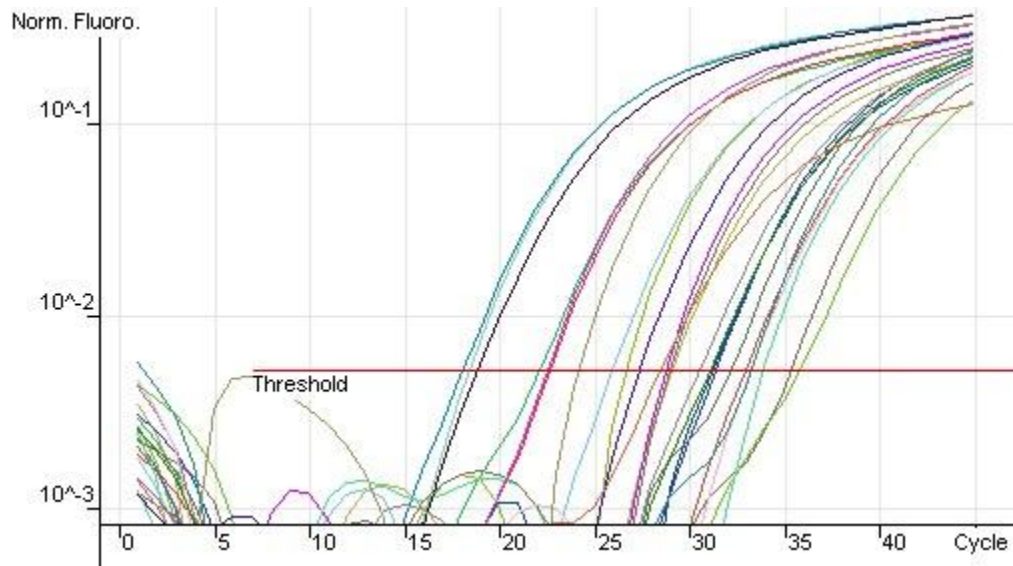
Cycle	Cycle Point
Hold @ 95°C, 10 min 0 secs	

Cycling (45 repeats)	Step 1 @ 95°C, hold 10 secs
	Step 2 @ 55°C, hold 45 secs, acquiring to Cycling A(Cy5,FAM/Sybr,JOE,ROX)

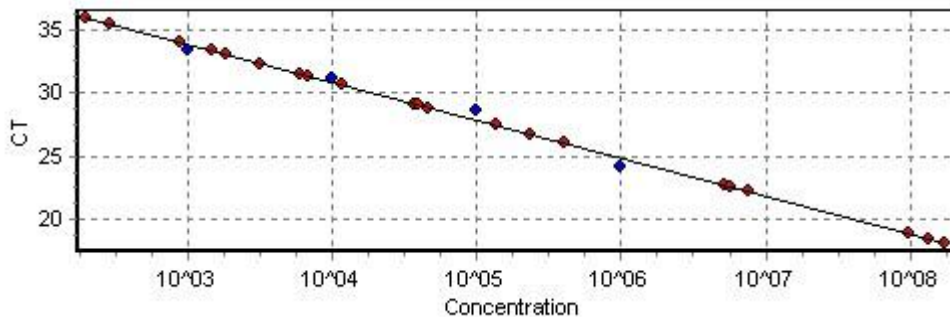
Raw Data For Cycling A.FAM/Sybr































Quantitation data for Cycling A.FAM/Sybr



Standard Curve



No.	Colour	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
1		a 10 ⁷	Unknown	18.47		134,150,356	
2		a 10 ⁶	Unknown	22.18		7,661,077	
3		a 10 ⁵	Unknown	26.71		233,984	
4		a 10 ⁴	Unknown	29.12		36,437	
5		a 10 ³	Unknown	31.29		6,834	
6		a 10 ²	Unknown	35.40		286	
7		10 ⁷ ctrl	Unknown	33.00		1,820	
8		ntc a	NTC	35.89		197	
9		b 10 ⁷	Unknown	18.14		172,928,256	
10		b 10 ⁶	Unknown	22.70		5,141,772	
11		b 10 ⁵	Unknown	26.00		401,524	
12		b 10 ⁴	Unknown	28.82		45,973	
13		b 10 ³	Unknown	31.44		6,073	
14		b 10 ²	Unknown	33.93		893	
15		10 ⁷ ctrl b	Unknown				
16		ntc b	NTC				
17		c 10 ⁷	Unknown	18.89		96,538,691	
18		c 10 ⁶	Unknown	22.58		5,642,803	
19		c 10 ⁵	Unknown	27.41		135,379	
20		c 10 ⁴	Unknown	29.01		39,632	
21		c 10 ³	Unknown	30.59		11,685	
22		c 10 ²	Unknown	32.29		3,156	
23		10 ⁷ ctrl	Unknown	33.28		1,476	
24		ntc 2	NTC				
25		10 ⁶	Standard	24.21	1,000,000	1,599,584	60.0%

No.	Colour	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
26		10 ⁵	Standard	28.55	100,000	56,368	43.6%
27		10 ⁴	Standard	31.14	10,000	7,690	23.1%
28		10 ³	Standard	33.31	1,000	1,442	44.2%

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(C)Corbett Research 2004

Control Program:

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Pump Movement,,Direction,Dispense,Volume,500,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 Pump Movement,,Direction,Aspirate,Volume,500,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 Pump Movement,,Direction,Dispense,Volume,500,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 Pump Movement,,Direction,Aspirate,Volume,500,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 Pump Movement,,Direction,Dispense,Volume,500,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 XYZ Movement,z = 5,X Position,No Movement,Y Position,No Movement,Z Position,Go to Position 5
 Pump Movement,,Direction,Dispense,Volume,350,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 Pump Movement,,Direction,Aspirate,Volume,350,Speed,Speed 1
 Wait Step,,Wait Time,5,,,
 Air Dry,Tip A,Tip to air dry,Tip A,Air dry time,10,,
 Air Dry,Tip B,Tip to air dry,Tip B,Air dry time,10,,
 Air Dry,Tip C,Tip to air dry,Tip C,Air dry time,10,,
 Air Dry,Tip D,Tip to air dry,Tip D,Air dry time,10,,
 Air Dry,Tip E,Tip to air dry,Tip E,Air dry time,10,,
 Air Dry,Tip F,Tip to air dry,Tip F,Air dry time,10,,
 Air Dry,Tip G,Tip to air dry,Tip G,Air dry time,10,,
 Air Dry,Tip H,Tip to air dry,Tip H,Air dry time,10,,
 XYZ Movement,z = 2,X Position,No Movement,Y Position,No Movement,Z Position,Go to Position 2

Bibliography

1. Coyne, S.R., et al., *Comparative Analysis of the Schleicher and Schuell IsoCode Stix DNA Isolation Device and the Qiagen QIAamp DNA Mini Kit*. J. Clin. Microbiol., 2004. **42**(10): p. 4859-4862.
2. Dauphin, L.A., B.D. Moser, and M.D. Bowen, *Evaluation of five commercial nucleic acid extraction kits for their ability to inactivate Bacillus anthracis spores and comparison of DNA yields from spores and spiked environmental samples*. Journal of Microbiological Methods, 2009. **76**(1): p. 30-37.
3. Queipo-Ortuño, M., et al., *Comparison of seven commercial DNA extraction kits for the recovery of Brucella DNA from spiked human serum samples using real-time PCR*. European Journal of Clinical Microbiology & Infectious Diseases, 2008. **27**(2): p. 109-114.
4. Boom, R., et al., *Rapid and simple method for purification of nucleic acids*. J. Clin. Microbiol., 1990. **28**(3): p. 495-503.
5. Karalus, R.J., D. R. Pawlowski, *Method and Device for Isolating a Protein Sample*. , U. 12/692194, Editor 2010, CUBRC, Inc.: United States USPTO.
6. Pepinsky, R.B., *Selective precipitation of proteins from guanidine hydrochloride-containing solutions with ethanol*. Anal Biochem, 1991. **195**(1): p. 177-81.
7. Pawlowski, D.R. and R.J. Karalus, *Electricity-Free, Sequential Nucleic Acid and Protein Isolation*. J Vis Exp, 2012(63): p. e4202.